

CLAIMS

1. A bioassay method for testing a sample of soil, sand, sediment or other particulate material for the presence of a contaminant, comprising: extracting an  
5 assayable amount of the contaminant from the particulate material into a water-miscible solvent capable of dissolving the contaminant; mixing the resultant solvent solution of the contaminant with water and optionally a surfactant, whereby a mixture is obtained containing water, solvent, surfactant (if present) and any extracted contaminant; and exposing an organism to the mixture under  
10 conditions in which the inhibition, by the contaminant, of a signal generated by the organism can be related to the presence of the contaminant in the mixture.
2. An assay according to claim 1, wherein the contaminant assayed comprises one or more polycyclic aromatic hydrocarbon (PAH), one or more  
15 organic pesticide, one or more petroleum hydrocarbon, or any combination thereof.
3. An assay according to claim 1 or claim 2, wherein the signal is the emission of light by a bioluminescent organism.  
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4. An assay according to any one of claims 1 to 3, wherein the signal is the emission of light from *Vibrio fischeri*.
5. An assay according to any one of the preceding claims, wherein the  
25 amount of water mixed with the solution of the contaminant is up to about 30 times the volume of the solvent solution of the contaminant being diluted.
6. An assay according to claim 5, wherein the amount of water mixed with the solution of the contaminant is between about 10 and about 25 times the  
30 volume of the solvent solution of the contaminant being diluted.

7. An assay according to claim 5, wherein the amount of water mixed with the solution of the contaminant is between about 12 and about 15 times the volume of the solvent solution of the contaminant being diluted.
- 5 8. An assay according to any one of the preceding claims, wherein the water and the surfactant are mixed with the solvent solution of the contaminant as an aqueous surfactant solution.
9. An assay according to any one of the preceding claims, wherein the water-  
10 miscible solvent and the surfactant are each substantially less toxic to the organism at the concentration obtained, after dilution, in the mixture to be presented to the organism, than is the contaminant at the concentration in which it is present in the same mixture.
- 15 10. An assay according to claim 9, wherein the water-miscible solvent and the surfactant are each substantially non-toxic to the organism at the concentration obtained, after dilution, in the mixture presented to the organism.
11. An assay according to any one of the preceding claims, wherein the water-  
20 miscible solvent comprises one or more organic alcohol.
12. An assay according to claim 11, wherein the organic alcohol, or at least one of the organic alcohols, contains two to eight carbon atoms.
- 25 13. An assay according to claim 11 or claim 12, wherein the organic alcohol, or at least one of the organic alcohols, is an alkyl mono-ol containing three to six carbon atoms.
14. An assay according to any one of the preceding claims, wherein the water-  
30 miscible solvent comprises iso-propanol.

15. An assay according to any one of the preceding claims, wherein the water-miscible solvent comprises dimethyl sulphoxide (DMSO).
16. An assay according to any one of the preceding claims, wherein the water-miscible solvent consists essentially of one or more organic alcohol, DMSO, or a mixture thereof.
17. An assay according to any one of the preceding claims, wherein the water-miscible solvent consists essentially of a mixture of an organic alcohol and DMSO in a volume ratio of alcohol:DMSO between about 25:75 and about 75:25.
18. An assay according to claim 17, wherein the volume ratio of alcohol:DMSO is about 60:40.
19. An assay according to any one of claims 16 to 18, wherein the organic alcohol is as defined in any one of claims 12 to 14.
20. An assay according to any one of the preceding claims, wherein the surfactant is selected from fatty alcohol ethoxylates, alkylamine ethoxylates, mixtures of fatty alcohol ethoxylates and alkylamine ethoxylates, polyoxyalkylene polymers, block copolymers of more than one polyoxyalkylene, and any combination thereof.
21. An assay according to any one of the preceding claims, wherein the surfactant comprises Biononex, Lutrol F68, Lutrol F127, or any combination thereof.
22. An assay according to any one of the preceding claims, wherein the surfactant is used at an amount of less than about 5% by weight relative to the water.

23. An assay according to any one of the preceding claims, wherein the surfactant is used in an amount of less than about 2% by weight relative to the water.
- 5 24. An assay according to any one of the preceding claims, wherein the diluted mixture containing the contaminant includes one or more additional components.
25. An assay according to claim 24, wherein the additional component, or at least one of the additional components, is a further solute.
- 10 26. An assay according to claim 25, wherein the further solute is sodium chloride.
- 15 27. An assay according to any one of the preceding claims, wherein the solvent solution of the contaminant, or at least that portion of the solvent solution of the contaminant that is to be used for the assay, is substantially separated from the particulate material before mixing with at least one of the water and the surfactant.
- 20 28. An assay according to any one of the preceding claims, wherein the extraction of the contaminant into the solvent, the mixing of the solvent solution with the water and the surfactant, or both, is carried out under vigorous agitation.
- 25 29. An assay according to any one of the preceding claims, wherein the conditions in which the inhibition, by the contaminant, of a signal generated by the organism can be related to the presence of the contaminant in the mixture comprise measurement of a corresponding signal generated by a similar organism in a reference mixture.
- 30 30. An assay according to claim 29, wherein the reference mixture corresponds to the mixture under assay, but omits the contaminant.

31. An assay according to claim 29 or claim 30, wherein the difference in the measured inhibition between the mixture under assay and the reference mixture is related to the concentration of contaminant in the mixture under assay or in the particulate material, by means of calibration data relating the measured signal to the concentration of contaminant in the mixture under assay and data concerning the amount of particulate material and the volume of the mixture.

32. An assay according to claim 31, wherein the measurement is sufficiently quantitative to enable the determination to be made as to whether the concentration of the contaminant in the particulate material is above or below a certain level.

33. An assay according to claim 32, wherein the certain level is a legally specified level.

34. Apparatus for performing an assay as defined in any one of the preceding claims, the apparatus comprising: a first container containing the water-miscible solvent capable of extracting an assayable amount of a contaminant from a sample of soil, sand, sediment or other particulate material when contacted therewith; a second container containing the surfactant, optionally in aqueous solution; a third container containing the organism; means for detecting the signal generated by the organism; means for relating the signal to the presence of the contaminant in the mixture; and instructions, and optionally guidance indicia on at least one of the containers, for performing the assay.

35. An apparatus according to claim 34, further comprising means for determining and displaying whether the contaminant is present above or below a certain concentration in the particulate material.

36. An apparatus according to claim 35, wherein the certain level is a legally specified level.

37. An apparatus according to claim 35 or 36, wherein the parts are present as a kit in a portable container therefor.

38. An apparatus according to any one of claims 34 to 37, comprising: the  
5 first, second and third containers, the third container containing a bioluminescent organism; at least one additional container; as part of the means for relating the signal to the contaminant, a luminometer and ports for receiving two of the containers for differential simultaneous bioluminescence measurements on a  
10 reference mixture and on a contaminant-containing mixture; optionally a sample tray or other support surface; a plurality of small volume (preferably about 0.1 to about 3 ml) measuring devices for liquids (e.g. pipettes and/or syringes), at least some of which are optionally marked to show 0.5ml volume; optionally at least one glove; a measuring device for the particulate sample; associated microprocessors and software for the luminometer, or data connection means to  
15 permit connection of the luminometer to a remote computer; and means as necessary, for establishing connection to ancillary equipment.

39. An apparatus according to claim 38, wherein in total at least seven containers are present.

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40. A composition comprising about 3% to about 5% by weight of at least one organic alcohol, about 2% to about 4% by weight dimethyl sulphoxide (DMSO), about 0.5% to about 1.5% by weight of a surfactant, and the balance water and optionally a salt and optionally additional components.

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41. A composition according to claim 40, consisting essentially of the said amounts of the at least one organic alcohol, the DMSO, the surfactant, and the water and, if present, the salt, with less than about 10% by weight of any additional components.

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42. A composition according to claim 40 or 41, wherein the organic alcohol is as defined in any one of claims 12 to 14.

43. A composition according to any one of claims 40 to 42, wherein the surfactant is as defined in any one of claims 20 to 23.

5 44. A composition according to any one of claims 40 to 43, wherein the salt, when present, comprises sodium chloride.

45. A composition according to any one of claims 40 to 44, wherein the salt, when present, is at a concentration of between about 1% and about 3% by weight.

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46. A composition according to any one of claims 40 to 45, further comprising one or more polycyclic aromatic hydrocarbon (PAH), one or more organic pesticide, one or more petroleum hydrocarbon, or any combination thereof.

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